

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(12) UK Patent Application (19) GB (11) 2 261 072 A (13)

(43) Date of A publication 05.05.1993

(21) Application No 9219928.0

(22) Date of filing 21.09.1992

(30) Priority data

(31) 4133643

(32) 11.10.1991

(33) DE

(71) Applicant

Bruker Analytische Messtechnik GmbH

(Incorporated in the Federal Republic of Germany)

Silberstreifen, D-7512 Rheinstetten 4,
Federal Republic of Germany

(72) Inventor

Alexander Kock

(74) Agent and/or Address for Service

W H Beck, Greener & Co

7 Stone Buildings, Lincoln's Inn,
London, WC2A 3SZ, United Kingdom

(51) INT CL⁵

G01R 33/46

(52) UK CL (Edition L)

G1N NGA NG46

U1S S1082

(56) Documents cited

GB 2179747 A

(58) Field of search

UK CL (Edition K) G1B BCH, G1N

INT CL⁵ G01R 33/46

Online databases: WPI

(54) NMR analysis of fat content in mixtures, using chemical drying

(57) Determination of the fat or oil content of substances with high water content, in particular of food, is accomplished with a low resolution NMR spectrometer. Prior to the NMR measurement, the substance is mixed with a chemical drying agent, preferably with CaO powder. The protons originally in free water are then rigidly bound and their short T2 time permits discrimination.

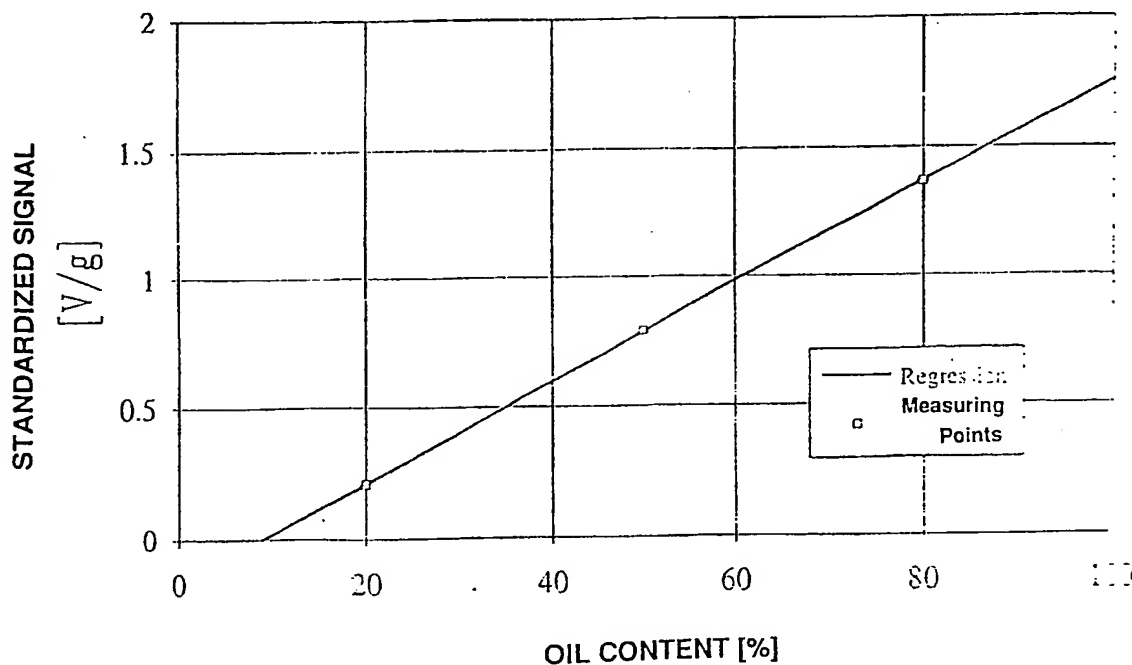


Fig. 2

GB 2 261 072 A

- 1/2 -

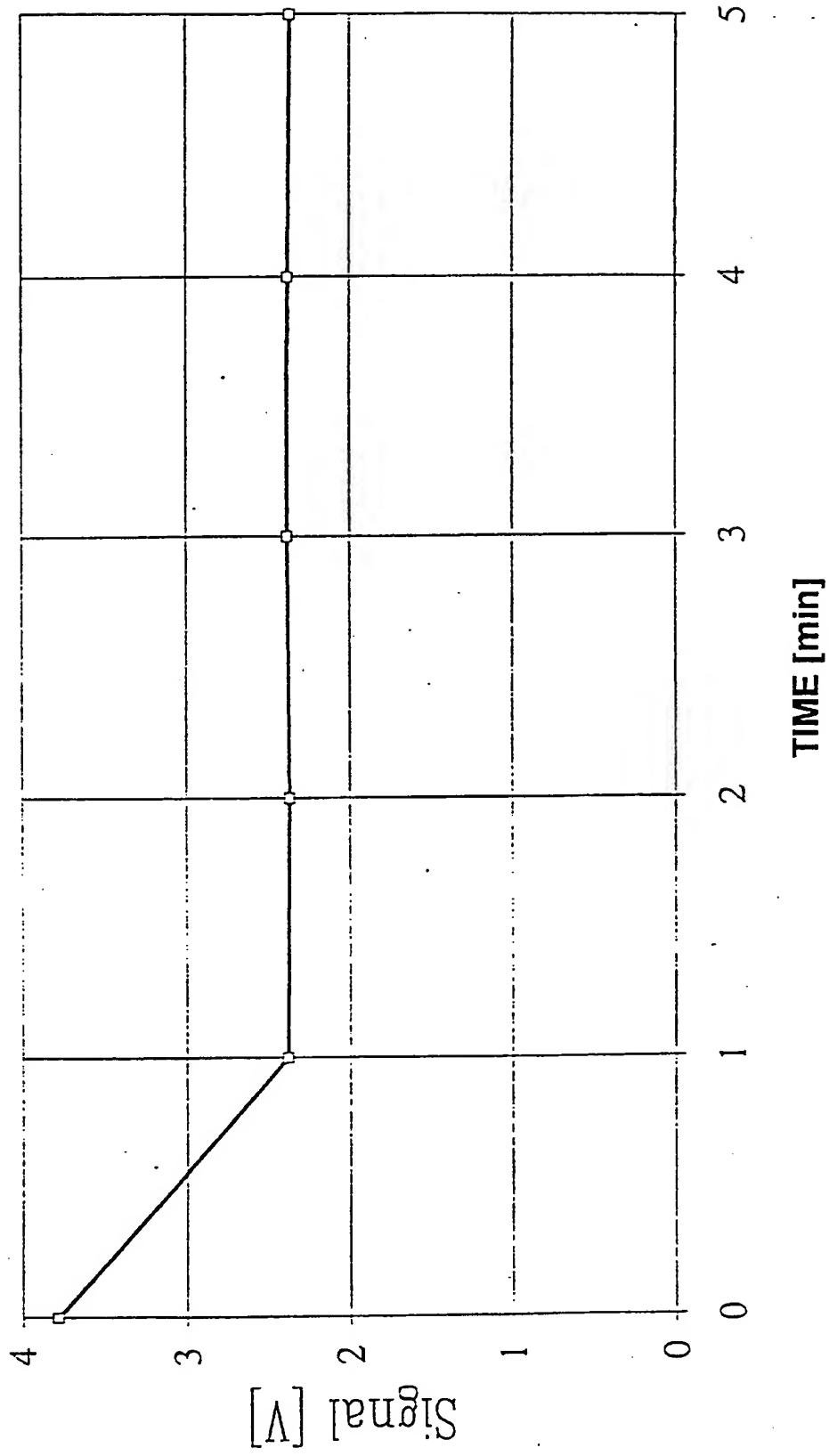


Fig. 1

- 2/2 -

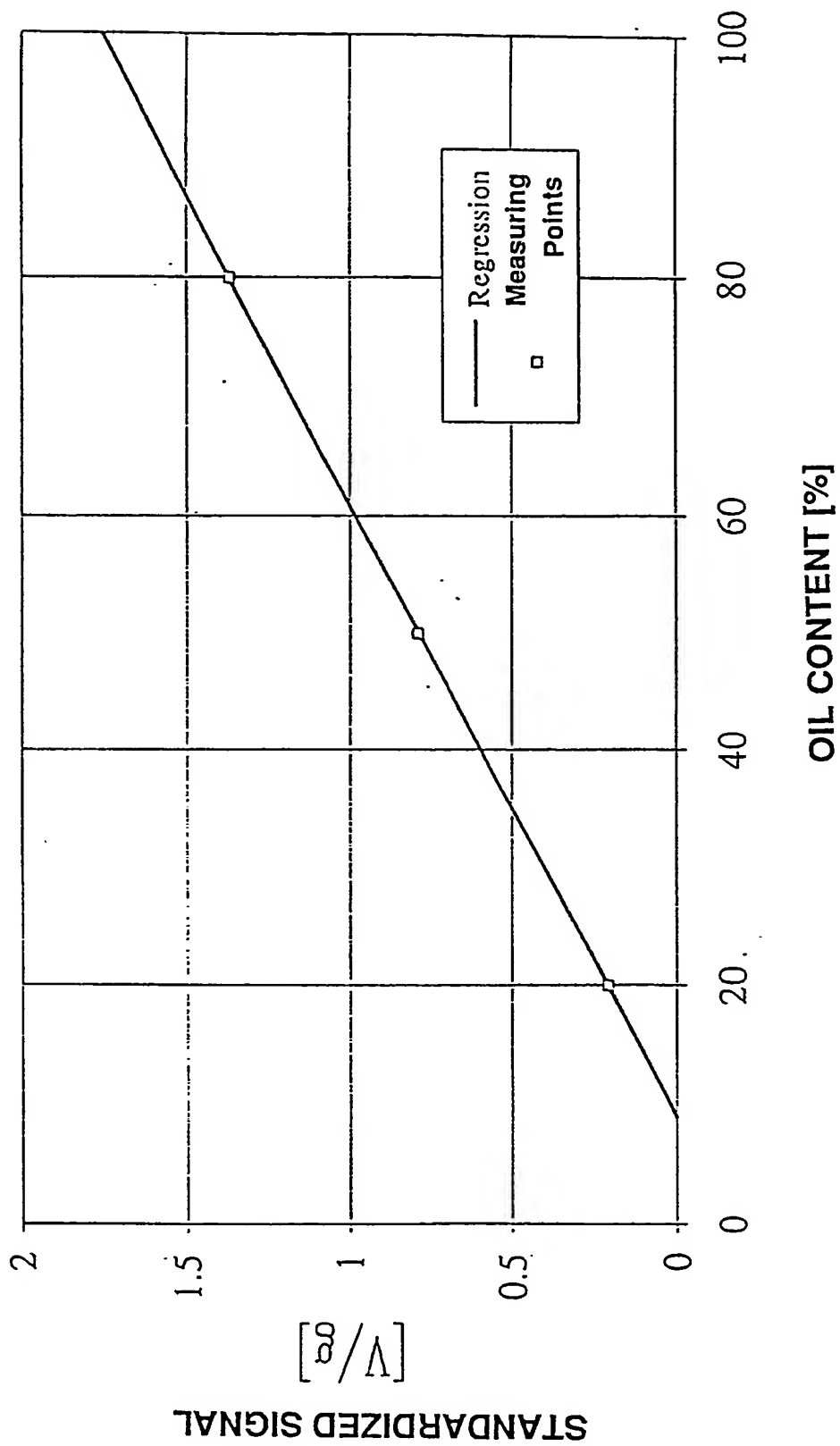


Fig. 2

Method of Determining Fat or Oil Content

5 The invention concerns a method to determine the fat or oil content of substances with high water content, in particular of food, by means of an NMR spectrometer.

10 Such a method is for example known from the article by H.Weisser in Zeitschrift für Lebensmitteltechnologie 28 (1977) 3, pages 97-101.

15 The determination of the fat content by means of a nuclear resonance pulse spectrometer of low spectral resolution with a magnetic field strength of maximum about 1 T and an average homogeneity of about 10^{-5} across the sample volume and as a result with a proton resonance frequency lower than about 50 MHz, e.g. the "MINISPEC" of the company BRUKER, 20 is not possible with products with a water content of, for example, more than 13 % because of the overlap of water and fat signals, since at a higher water content the contribution of the water to the total signal increases highly overproportionally. 25 Thus, determination of such products in their native state by means of nuclear resonance is limited to high resolution devices, which are, however, much too expensive for this purpose alone.

30 For low moisture contents in the range of a few percent a low resolution device, too, yields reliable results, since in this case the contribution of the water signal has practically completely died away after about one millisecond and as a result it 35 is possible to measure only the fat or oil content, respectively. By combination of two measurements at

different times after the excitation pulse it is even possible, also to measure the moisture content quantitatively apart from the fat content (see the articles of S.A.Jones in BRUKER Minispec Application Note 17 (1) 5 1983, P.J.Barker in BRUKER Minispec Application Note 32 (2) 1986 and P.N.Tiwari and W.Burg in J.American Oil Chemist's SOC. 57, No. 3, pages 119-121 (1979)). However, this method fails with larger water contents, since these have spin-spin relaxation times T_2 comparable to those of the 10 fat content and therefore the signal contributions can no longer be practically separated.

In order to perform such determinations with a low resolution device, it has been necessary hitherto to 15 eliminate the water by pre-drying in an oven, for example in a drying oven, a vacuum oven, a microwave oven or the like. The need for such drying apparatus and the time needed are both disadvantages of this method.

20 In accordance with the present invention, water is removed from such a sample at least partly by means of chemical drying.

By the chemical removal of the water, and as a consequence, 25 the overlapping water signal, the signal caused by the fat can be measured quickly,

easily and unambiguously. In this respect it is irrelevant whether the water has been removed completely. It is only important that the water content after the chemical drying is below a given
5 threshold. This threshold depends on the fraction of water which is rigidly bound inside the substance. Experimentally, about 13 % has been found to be a usable threshold.

10 A preferred chemical drying agent should fulfill the following criteria as far as possible:

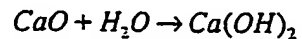
1. Neither it nor its reaction product should
15 give any NMR signal within the measuring range.
2. Both it and its reaction product should not be poisonous and should be tolerable for the environment.
- 20 3. It should quickly bind as much water as possible.
4. The reaction heat released must not cause any danger for the personnel.
- 25 5. It should be inexpensive.

A chemical which largely shows these qualities is
30 calcium carbide. The reaction product is the easily inflammable acetylene. Therefore one should work under a flue if using calcium carbide. This is also required for the additional reason that due to
35 impurities there is the danger that in addition to acetylene PH_3 is also formed, which at the least

has an unpleasant smell and which is also poisonous in higher concentrations.

5 In a particularly preferred method calcium oxide (CaO) is used as a drying agent. This substance fulfills all five requirements without the disadvantages of calcium carbide.

10 The basic reaction with water may be expressed as follows:



15 The heat released during this reaction is not sufficient to be dangerous. But it is sufficient to considerably heat up a sample of average water content starting from room temperature so that, if required, only a short time of tempering is needed in order to reach the necessary measuring tempera-
20 ture for the determination. This temperature is usually 40°C.

The invention is explained in detail with reference to the following example.

25 Obviously, the features that have been described above and will be explained hereafter can be used not only in the described combination but also in any other combination or individually without leaving the scope
30 of the present invention.

Usually, in a first step the spectrometer is calibrated by means of substances of known fat content.
35 The time schedule and quite generally the measuring parameters should largely be identical to those of

the subsequent measurement. The following procedure has proved to be advantageous. A glass tube of for example 13 mm length is filled to just below half its length with the calibration sample of known fat con-

5 tent and the weight of the contents is recorded. CaO powder of about the same mass is added so that the tube is not quite filled. This ratio of masses has turned out to be appropriate for a wide range of mayonnaise samples with water contents of 17 %
10 to 65 %. The amount of added CaO must be adapted to the maximum expected water content (e.g. 65 %) and should be chosen such that the corresponding amount of water can be completely converted together with the CaO. A surplus of CaO is on its own not harmful
15 but only worsens the filling factor of the measurement. However, the CaO content must at least be sufficient to reduce the water content after the reaction below the threshold of for example 13 %.

20 After the reaction with water the total volume increases because of the lower density of calcium hydroxide so that the standard filling level of about 30 mm is approximately reached. The mixture is carefully stirred with a spatula. Material still
25 sticking to the spatula is wiped off with paper. The paper should be dry so that in the measuring range no additional water signal is produced. The paper is pressed onto the sample inside the sample tube and stays inside the tube. After
30 this, the sample has heated up noticeably and is only brought to measuring temperature in a short additional tempering step. However, this tempering step can also be omitted.

35 The proton NMR signal is then measured and correlated by means of a table to the known fat

content. This calibration procedure is performed with several (in general at least three) calibration substances. This yields a correlation between NMR signal, standardized to the mass of the calibration sample, and the fat content which has to be interpolated inside the range of interest. This correlation is stored in the computer of the spectrometer, e.g. in the form of a table or analytically. It should be emphasized that the calibration, assuming a sufficient amount of CaO, is independent of the water contents of the calibration samples, just as the eventual measurements should be independent of the water contents of the measuring samples.

A calibration, once established, can be used for a long time, as long as nothing changes with respect to the measuring procedure, to the apparatus, and to boundary conditions. It goes without saying that the calibration should periodically be checked.

During the subsequent measurement of unknown products one has to proceed in the same way. However, the measured NMR signal, standardized to the sample mass, is compared to stored calibration tables or calibration curves, respectively, and from this a fat content is derived which is displayed.

The measuring sequence for the calibration and the measurement is usually a spin echo sequence, i.e. an excitation high frequency pulse (generally a 90° pulse) is after a delay period in the millisecond range, e.g. after three milliseconds, followed by a second pulse usually a 180° pulse phase shifted by 90° . After again the same delay time of e.g. further three milliseconds a spin echo can be detected. For this purpose

it is sufficient to take one measuring point at the known point of time of the occurrence of the spin echo. Details of the pulse sequences may be found in textbooks on nuclear resonance. A spectrometer with which the invention can be performed is described in the brochure "Minispec PC 100" (September 1990) of the company BRUKER Analytische Meßtechnik GmbH. To improve the signal-to-noise ratio several echoes can be produced and accumulated by repeated 180° pulses, or the measurement can be repeated and accumulated after a delay time which is large compared to the maximum spin-lattice relaxation time of the sample (several seconds). The spin echo method is particularly advantageous since at the point of time of the echo, e.g. after six milliseconds, the signal contributions of rigidly bound water molecules, e.g. in lipid form, with correspondingly short spin-spin relaxation times T_2 are already decayed. Obviously after the drying procedure leading down to a water content threshold of e.g. 13 % and below, predominantly bound H_2O molecules remain so that the contribution of the water signal to the spin-echo signal has decayed overproportionally to a negligible value. This result has been confirmed experimentally for a multitude of mayonnaise types, but it is also true for other solid-liquid mixtures (see for example the above mentioned article by H. Weisser).

The calibration and the measurement require a time of less than five minutes per sample, therefore we are dealing here with a fast method.

Other fast methods (e.g. the Foss-Let-Method; A.Montag in Deutsche Lebensmittelrundschau, Volume

69, pages 470 ff., 1973: "Zur densitometrischen Fettbestimmung mit dem Foss-Let-Gerät") require at least 15 minutes and moreover use chemicals which are far more hazardous.

5

The intention of the figure is to show how the chemical drying procedure as a function of time is reflected in the spin-echo amplitude (Fig. 1) and how for a number of calibration substances the spin echo amplitude (in the dried state) depends on the fat content (Fig. 2).

10

In detail, Fig. 1 shows the time dependence of the spin-echo amplitude after mixing with CaO for a typical mayonnaise. A spin-echo sequence with 90° excitation pulse, a delay time of three milliseconds and a 90° phase shifted 180° pulse were used so that the spin echo occurred six milliseconds after the 90° pulse. For every measuring point shown the sequence was repeated three times and accumulated.

15

20

At first the amplitude decreases quickly and noticeably, i.e. water is extracted and converted together with the CaO into Ca(OH)_2 . Now this part can no longer contribute to the signal. However, it can be seen that already after one minute the drying procedure is completed insofar as it does not effect a further decrease in the signal, i.e. the water concentration has decreased so far that the water which may not yet have reacted with the CaO is bound in the mayonnaise in such a way that because of its short T_2 time it cannot yield a noticeable contribution to the spin echo (after six milliseconds).

25

30

35

Fig. 2 shows the amplitude of the spin-echo signal for three calibration mayonnaises with fat contents 20%, 50% and 80%, standardized to one gram of mayonnaise. The three measuring points lie very well on a straight line, which, however, does not exactly go through the origin. Therefore, in the range from 20% to 80% one can simply interpolate linearly. On the other hand, it is obvious that for smaller concentrations, the calibration measurement cannot simply be extrapolated linearly. Either one has to restrict oneself to the explicit range covered or the calibration has to be completed by additional calibration points in order to describe the non-linear range to a sufficient extent.

Claims

1. A method for determining the fat or oil content in a sample with a high water content by means of a NMR pulse spectrometer, wherein the substance is dried prior to measurement by mixing it with a chemical drying agent.

2. A method as claimed in Claim 1, wherein the chemical drying agent is calcium carbide.

3. A method as claimed in Claim 1, wherein the chemical drying agent is CaO.

4. A method as claimed in any one of the preceding claims, wherein the NMR spectrometer has a maximum proton resonance frequency of 50 MHz and a magnetic field inhomogeneity across the measuring volume of at least 10^{-5} .

5. A method as claimed in any one of the preceding claims, wherein the NMR spectrometer is used to measure a proton spin echo signal of the sample.

6. A method as claimed in Claim 5, wherein the proton spin-echo signal of at least one calibration substance with known fat or oil content is measured prior to the measurement of the spin-echo signal of the sample and the fat or oil content of the sample is derived by comparison of the spin-echo amplitudes of calibration and sample measurement.

7. A method as claimed in any one of the preceding claims, comprising the steps of:-

a. mixing a known amount of a calibration substance of known fat or oil content with an amount of a chemical drying agent sufficient to reduce the water content of the

calibration substance below a desired threshold,

b. carrying out a nuclear resonance measurement of the reacted mixture;

5

c. determining the amplitude of the nuclear resonance signal;

d. standardizing this amplitude to the amount of the calibration substance;

10

e. recording the value of the standardized amplitude;

f. optionally, repeating the steps a. to e. for a plurality of calibration substances;

15

g. computing a calibration table or a calibration curve from the standardized amplitudes of step e., which give the correspondence between fat or oil content and standardized amplitude of the nuclear resonance signal;

20

h. repeating steps a. to e. replacing the calibration substance by a sample of unknown fat or oil content;

i. computing the fat or oil concentration, respectively of the sample, from the measured standardized amplitude of the nuclear resonance signal and the calibration table or calibration curve, respectively;

25

j. displaying or storing the fat or oil concentration, respectively, for the sample.

30

k. optionally, repeating the steps h. to j. for a plurality of samples;

35

8. A method as claimed in any one of the preceding claims,

wherein the sample is a mayonnaise.

9. A method of determining fat content of a sample, substantially as hereinbefore described.

- 13 -

Patents Act 1977
Examiner's report to the Comptroller under
Section 17 (The Search Report)

Application number

GB 9219928.0

Relevant Technical fields

(i) UK Cl (Edition L) G1N; G1B (BCH)

(ii) Int Cl (Edition 5) G01R (33/46)

Search Examiner

K SYLVAN

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI

Date of Search

17 DECEMBER 1992

Documents considered relevant following a search in respect of claims 1 TO 9

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
A	GB 2179747 A (PICKER) see whole document	-

Category	Identity of document and relevant passages	Relevant to claim(s).

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family, corresponding document.

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).